MICROBIOLOGICAL ASSAYS FOR *p*-AMINOBENZOIC ACID

Sirs:

We have developed in this laboratory a microbiological assay for *p*-aminobenzoic acid, using a mutant strain of *Neurospora crassa*[1] very generously supplied to us by Dr. G. W. Beadle of Stanford University. Application of this method, based on studies by Lampen, Underkofler, and Peterson,[2] to aqueous extracts of various natural substances yields results which suggest that the assay procedure of Landy and Dicken[3] responds to

TABLE I

Substances	*p*-Aminobenzoic acid	
	Hot water extract	Acid hydrolysis
	γ per gm. moist tissue	*γ per gm. moist tissue*
Beef liver	<0.1	2.5
Spinach	0.12	0.6
Oats (seed)	0.13	0.5
Mushrooms	0.5	1.3
Fresh yeast	3.6	4.0

TABLE II

	H_2SO_4	*p*-Aminobenzoic acid
	N	*γ per gm.*
1 hr. autoclaving, 15 lbs.	0.1	0.9
	1.0	1.2
	6.0	2.2
	12.0	2.0

only a fraction of the total amount of *p*-aminobenzoic acid obtainable after acid or alkaline hydrolysis. We find, moreover, that enzymatic hydrolysis[4] or autolysis[5] is not always sufficient to lead to the maximum effect. The increased yield obtainable by heating with 6 N H_2SO_4 for 1 hour in the autoclave at 115.5° is demonstrated by the examples in Table I.

[1] Tatum, E. L., and Beadle, G. W., *Proc. Nat. Acad. Sc.*, **28,** 234 (1942).

[2] Lampen, J. O., Underkofler, L. A., and Peterson, W. H., *J. Biol. Chem.*, **146,** 277 (1942).

[3] Landy, M., and Dicken, D. M., *J. Biol. Chem.*, **146,** 109 (1942).

[4] Cheldelin, V. H., Eppright, M. A., Snell, E. E., and Guirard, B. M., *Univ. Texas Pub. 4237* (1942).

[5] Blanchard, K. C., *J. Biol. Chem.*, **140,** 919 (1941).

The effect of acid concentration on the yield of the vitamin from beef liver is shown in Table II.

Recently in this laboratory Dr. D. E. Pennington has developed an extremely sensitive microbiological assay method for *p*-aminobenzoic acid, using a lactic acid bacterium as the test organism. A comparison of assay results by this and the *Neurospora* method is in progress and complete details will be published in the near future.

Biochemical Institute and the Clayton Foundation for Research
The University of Texas
Austin

HERSCHEL K. MITCHELL
EDITH R. ISBELL
ROY C. THOMPSON

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